monolayers could be induced to undergo cell fate transitions. Growth in neural differentiation medium caused the cells to acquire an anterior/eye field progenitor marker profile and suppress expression of the RPE marker MITF. Surprisingly, the authors also found that growth in media promoting differentiation toward mesenchymal lineages resulted in expression of markers consistent with adipocyte, chondrocyte, and osteogenic phenotypes. To rule out the possibility that these cell types were generated by contaminating mesenchymal cells present in the starting culture, the authors expanded clonal RPESC lines and confirmed that they were capable of generating RPE and mesenchymal progeny. Furthermore, they found that GFP-labeled human RPESCs could also give rise to mesenchymal derivatives in a chick chorioallantoic membrane assay.

Cellular plasticity in the RPE has been regarded primarily as a property of lower vertebrates (Araki, 2007). However, these new findings suggest that adult human RPESCs may hold the potential to undergo complete transdifferentiation to neuroretinal and other phenotypes. As the authors discuss, an innate capacity of the RPE to dedifferentiate in vivo leads to a pathological condition called proliferative vitreoretinopathy (PVR). PVR occurs in the eye when the monolayer of RPE is disrupted, typically by detachment of the overlying neural retina. RPE cells dislodge from their underlying Bruch's membrane and proliferate along a mesenchymal lineage, resulting in a fibroblastic scar (Figure 1). Salero and colleagues were able to recapitulate this phenomenon in vitro, providing an important tool for identifying therapeutics that can inhibit this process. Equally, understanding how dedifferentiation and expansion of RPE cells is regulated may help in other diseases that involve RPE degeneration.

Last but not least, these results provide an important illustration of the reprogramming capacity of not just induced pluripotent stem cells but even adult somatic cells. Salero et al. convincingly show that RPE cells maintain the ability in adulthood to reprogram to become multipotent along a mesenchymal lineage and form precursors of a number of different cell types. They therefore represent an additional source of adult human stem cells.

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Tales From the Crypt: The Expanding Role of Slow Cycling Intestinal Stem Cells

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DOI 10.1016/j.stem.2011.12.012

Similar to other highly self-renewing tissues, the intestinal epithelium contains both slowly and rapidly cycling progenitor/stem cells, though their relationship has been largely unexplored. Two recent reports in *Nature* (Tian et al., 2011) and *Science* (Takeda et al., 2011) shed new light on their dynamic interplay.

The small intestinal epithelium has enormous capacity for self-renewal, replacing itself every 3 to 5 days. The cellular basis for this regenerative potential has long been accepted to reside in multipotent intestinal stem cells (ISCs) (Cheng and Leblond, 1974). Based on the hypothesis that ISCs would be slowly cycling, Potten and colleagues initially employed DNA label retention models to identify these cells. These studies led to the discovery and characterization of putative ISCs located in the "+4 crypt position" (Figure 1) (Potten et al., 1974). While this finding subsequently gave rise to the discovery of a number of additional markers based on colocalization with label retention, functionally validated ISC markers remained elusive for over three decades (reviewed in Montgomery and Breault, 2008).

The first functionally validated ISC marker to be identified was *Lgr5*, a downstream target of canonical Wnt signaling (Barker et al., 2007). In contrast to Potten's original observation, *Lgr5* expression corresponded to crypt base columnar cells, located between Paneth cells at the crypt base (Figure 1), a site previously suggested to contain ISCs (Bjerknes and Cheng, 1999). Surprisingly, and in stark contrast to the label-retaining population, the majority of *Lgr5*-expressing cells were shown to be rapidly cycling, raising doubts as to whether bona fide slowly cycling ISCs were also present in

this highly self-renewing tissue. On the other hand, as with other rapidly cycling cells, this population has been shown to be highly sensitive to the effects of intestinal damage (Barker et al., 2007), strongly suggesting that an alternative system would be required to restore homeostasis following injury. Moreover, how such a rapidly cycling population could be maintained as the solitary stem cell for the life of the organism, without accumulating deleterious mutations, has been the subject of much debate.

In support of Potten's initial hypothesis, the intestinal stem cell field has recently witnessed the emergence of functional evidence establishing that slowly cycling, label-retaining ISCs exist within the intestinal crypt and are distinct from *Lgr5*-expressing cells. These cells, marked by *Bmi1* (Sangiorgi and Capecchi, 2008) and *mTert* expression (Montgomery et al., 2011), are largely quiescent (or presumed to be in the case of *Bmi1*-expressing cells) and are located predominantly in the "+4 crypt position." Under steady-state conditions, slowly cycling stem cells contribute to intestinal lineage development largely through an *Lgr5* cell-dependent pathway (Figure 1A, bold red arrow). In addition, a second lineage pathway, which is *Lgr5* cell-independent, also mediates cell fate decisions, albeit less frequently (Figure 1A, thin blue arrow) (Montgomery et al., 2011). These discoveries raise important questions regarding the cellular plasticity of daughter cells and the mechanisms regulating their fate decisions. Intriguingly, slowly cycling stem cells are highly resistant to intestinal injury and play an important role during intestinal regeneration (Figures 1B and 1C) (Montgomery et al., 2011), perhaps by restoring the rapidly cycling ISC population. Together, these observations suggest that regulation of intestinal homeostasis may be similar to other highly self-renewing tissues, e.g., blood (Wilson et al., 2008) and skin (Fuchs, 2009), which are maintained by slowly cycling stem cell populations capable of restoring homeostasis following loss of rapidly cycling progenitor cells. Definitive evidence to support this claim, however, has been lacking.

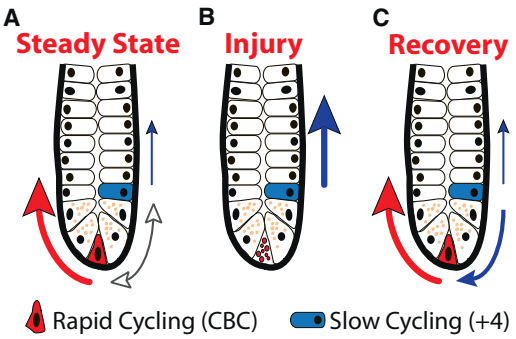

Figure 1. Role of Slowly and Rapidly Cycling Intestinal Stem cells

(A) Intestinal crypt under steady-state conditions showing interconversion of slowly and rapidly cycling stem cells (bidirectional arrow) as well as the dominant contribution of rapidly cycling stem cells (bold red arrow) and minor contribution of slowly cycling stem cells in the +4 position (thin blue arrow).

(B) Upon injury, rapidly cycling stem cells undergo apoptosis forcing slowly cycling stem cells to assume a dominant role (blue arrow).

(C) During recovery from injury, slowly cycling stem cells give rise to the rapidly cycling population restoring homeostasis. Paneth cells, marked by dense granules, define the niche boundaries.

Two recent reports provide essential new insight into the interdependence of slowly and rapidly cycling ISC populations. Tian and colleagues employed lineage tracing and cell ablation studies using two newly generated mouse strains targeting the *Lgr5* locus (*Lgr5-dsRED-IRES-CreERT2* and *Lgr5-EGFP-DTR*). In a series of elegant studies, they report that elimination of the rapidly cycling ISC population had no effect on intestinal homeostasis, leading them to conclude that *Lgr5*-expressing cells are dispensable. They go on to demonstrate that *Bmi1*-expressing cells are dramatically increased in number following *Lgr5* cell ablation and function as a reserve stem cell pool contributing to intestinal lineage development via the *Lgr5* cell-independent pathway (Figure 1B). Of note, upon removal of the ablation signal, *Bmi1*-expressing cells rapidly give rise to *Lgr5*-expressing cells, thereby restoring the *Lgr5* cell-dependent lineage pathway (Figure 1C). Finally, they demonstrate that, like *mTert* (Montgomery et al., 2011), *Bmi1*-expressing cells can contribute to *Lgr5*-expressing cells under steady-state conditions, further supporting the concept that slowly cycling ISCs restore rapidly cycling cells (Figure 1A).

Extending these observations, Takeda and colleagues identified *Hopx* gene expression as another marker of slowly cycling ISCs at the "+4 crypt position." In contrast to the rare nature of *Bmi1*-expressing ISCs and the rarer *mTert* population, *Hopx*-expressing cells appear to be present in virtually every crypt, raising the possibility that functionally distinct subpopulations of slowly cycling ISCs exist. Through a series of well-designed lineage-tracing studies using a newly generated mouse strain targeting the *Hopx* locus (*Hopx-CreER*), they demonstrate that slowly cycling *Hopx*-expressing ISCs give rise to *Lgr5*-expressing ISCs, consistent with *Bmi1* and *mTert* ISCs. Importantly, however, they demonstrate for the first time that *Lgr5*-expressing cells can contribute to *Hopx*-expressing cells, data that further underscore that a dynamic relationship exists between distinct ISC populations under steady-state conditions (Figure 1A). Whether a similar relationship exists with regenerative pressure remains unclear. However, given that *Lgr5*-expressing cells are highly sensitive to injury (Barker et al., 2007) and that slowly cycling ISCs utilize an *Lgr5*-independent pathway following injury to regenerate the crypt (Figure 1b), it is unlikely this interchange is functionally important under all conditions. Although Takeda et al. show that interconversion occurs between slowly and rapidly cycling ISCs, the precise mechanisms underlying this cellular plasticity remain to be determined.

Taken together, these two reports provide fundamental new insight into the dynamic role stem cells play in the intestinal crypt. These investigators demonstrate that rapidly and slowly cycling ISC populations are interdependent and work cooperatively to maintain intestinal homeostasis under both steady state and regenerative conditions (Figure 1). The development of these and other mouse model systems will ultimately give rise to a detailed understanding of the lineage relationships and hierarchy among the various ISC populations within the crypt. Along these lines, it will be interesting to determine whether the principles governing intestinal lineage development are analogous to those involved in other stem cell systems. For example, the hematopoietic

system is maintained by stem cells with long- and short-term regenerative potential, which are predominantly quiescent and resistant to injury, as well as committed multipotent, oligopotent, and unipotent progenitors, which have increased proliferative potential. Deciphering how environmental and physiological inputs regulate signaling pathways affecting slowly and rapidly cycling ISC populations in the niche during homeostasis and pathological states such as cancer and inflammatory bowel disease will be important directions for future studies.

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Cbx Proteins Help ESCs Walk the Line between Self-Renewal and Differentiation

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DOI 10.1016/j.stem.2011.12.011

The Polycomb repressive complexes (PRC) regulate self-renewal and differentiation in embryonic stem cells (ESCs). In this issue of *Cell Stem Cell*, Morey et al. (2012) and O'Loghlen et al. (2012) report that dynamic interchange of PRC subunits modulates the balance between self-renewal and lineage commitment in ESCs.

Differentiate or self-renew? This is the principal question faced by all stem cells. The self-renewal of embryonic stem cells (ESCs) is maintained through expression of pluripotency genes and repression of lineage-specific genes. Conversely, differentiation is achieved through repression of the genes required for pluripotency, with simultaneous activation of a cascade of lineage-specific epigenetic and transcriptional changes. First identified in *Drosophila*, the Polycomb group (PcG) proteins are known regulators of ESC differentiation and do so by maintaining repressive chromatin states. The mammalian genome encodes multiple homologs of PcG components which broadly associate in two functionally distinct complexes, PRC1 and PRC2. PRC2 has been shown to functionally trimethylate lysine 27 on histone H3 (H3K27me3), while PRC1 monoubiquitylates histone H2A on lysine 119 (H2AK119Ub1) (Cao and Zhang, 2004; de Napoles et al., 2004). The prevailing dogma posits PRC2 and PRC1 work as a team to prevent transcription of genes that initiate differentiation. Mechanistically this is thought to occur via a PRC2-mediated deposition of H3K27me3 followed by H2AK119Ub1 catalyzed by PRC1 specifically at these sites. In pluripotent cells, PRC1/PRC2 co-occupy regions which overlap with H3K27me3, and a large proportion of these sites are proximal to genes involved in development and lineage commitment (Ku et al., 2008). Additionally, loss of function of either PRC1 or PRC2 in pluripotent cells does not affect expression of key pluripotency genes, but rather leads to derepression of genes normally upregulated during differentiation (Chamberlain et al., 2008; Leeb and Wutz, 2007).

Although PRC1 functionally targets PRC2 modified chromatin, it is unclear how PRC1 identifies sites of PRC2 catalyzed H3K27me3 and what regulatory mechanisms exist to facilitate derepression of PcG bound chromatin in response to ESC differentiation. Unlike PRC2, the PRC1 complex has been shown to contain a number of Polycomb orthologs (PCs) known as the Cbx family of proteins. Cbx proteins have been shown to interact directly with methylated histone H3 and are enriched at sites of heterochromatin (Bernstein et al., 2006). While PRC1 is functionally important for ESC self-renewal and differentiation, there has been no clear experimental evidence linking Cbx proteins with PRC1